24jul01 11:41:34 User217743 Session D534.1
$0.00 0.174 DialUnits FileHomeBase
$0.00 Estimated cost FileHomeBase
$0.00 Estimated cost this search
$0.00 Estimated total session cost 0.174 DialUnits
File 410:Chronolog(R) 1981-2001/June
(c) 2001 The Dialog Corporation

Set Items Description
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? set hi *;set hi *

HILIGHT set on as '*'*
*HILIGHT set on as '*'
? b 155

24jul01 11:41:40 User217743 Session D534.2
$0.00 0.056 DialUnits File410
$0.00 Estimated cost File410
$0.00 Estimated cost this search
$0.00 Estimated total session cost 0.229 DialUnits
File 155:MEDLINE(R) 1966-2001/Jul W5
(c) format only 2001 Dialog Corporation
*File 155: This file has been reloaded. Accession numbers have changed. Please see Help News155 for further details.

Set Items Description
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? s glycoprotein()hormone and single()chain

59620 GLYCOPROTEIN
175585 HORMONE
743 GLYCOPROTEIN(W)HORMONE
428007 SINGLE
252892 CHAIN
3539 SINGLE(W)CHAIN
S1 10 GLYCOPROTEIN()HORMONE AND SINGLE()CHAIN
? t s1/free

1/8/1
DIALOG(R)File 155:(c) format only 2001 Dialog Corporation. All rts. reserv.
11221856 21150064 PMID: 11250932
High-level expression of a functional *single*-*chain* human chorionic gonadotropin-luteinizing hormone receptor ectodomain complex in insect cells.
Apr 2001
Tags: Animal; Human; Support, U.S. Gov't, P.H.S.
Descriptors: *Insects--metabolism--ME; *Receptors, LH--biosynthesis--BI; Cells, Cultured; Cloning, Molecular; Electrophoresis, Polyacrylamide Gel; Epitopes--genetics--GE; Gonadotropins, Chorionic--metabolism--ME; Iodine Radioisotopes--diagnostic use--DU; Receptors, Cell Surface--metabolism--ME; Receptors, LH--genetics--GE; Receptors, LH--isolation and purification--IP ; Recombinant Proteins--biosynthesis--BI; Recombinant Proteins--genetics --GE; Reverse Transcriptase Polymerase Chain Reaction
CAS Registry No.: 0 (Epitopes); 0 (Gonadotropins, Chorionic); 0 (Iodine Radioisotopes); 0 (Receptors, Cell Surface); 0 (Receptors, LH) ; 0 (Recombinant Proteins); 0 (effector cell protease receptor-1) ? t s1/3,ab/all

1/3,AB/1
DIALOG(R)File 155:MEDLINE(R)
(c) format only 2001 Dialog Corporation. All rts. reserv.

11221856 21150064 PMID: 11250932
High-level expression of a functional *single*-*chain* human chorionic gonadotropin-luteinizing hormone receptor ectodomain complex in insect cells.
Fralish GB; Narayan P; Puett D
Department of Biochemistry and Molecular Biology, University of Georgia, Athens, Georgia 30602-7229, USA.
Endocrinology (United States) Apr 2001, 142 (4) p1517-24, ISSN 0013-7227 Journal Code: EGZ
Contract/Grant No.: DK-33973, DK, NIDDK
Languages: ENGLISH
Document type: Journal Article
Record type: Completed
Reproductive capacity in primates is dependent on the high-affinity binding of the glycoprotein hormones LH and human (h)CG to the large ectodomain (ECD) of their common receptor (LHR). Our understanding of the precise molecular determinants of hormone binding is limited, because there are no structural data for any of the *glycoprotein* *hormone* receptors. Overexpression of the ECD of the receptor has been attempted in various expression systems. Prokaryotic expression does not yield properly folded ECD. Eukaryotic expression, on the other hand, results in mostly heterogeneous, intracellularly trapped protein, but the secreted ECD is completely folded. Accordingly, we have tethered the *single*- *chain* hormone, yoked hCG, to the N terminus of LHR-ECD (yoked hormone-extracellular domain). Yoked hCG is secreted at high levels; binds LHR with high affinity; and, when tethered to the N terminus of full-length LHR, it binds and constitutively activates the receptor. Using recombinant baculovirus, yoked hormone-extracellular domain is secreted from insect cells at levels greater than 1 microg/ml, nearly 20-fold higher than that previously reported in eukaryotic expression systems. The protein was purified and binds exogenous (125)I-hCG with high affinity but, significantly, only after protease treatment to remove the tethered hormone. Thus, the fusion protein seems to form

a functional hormone-receptor complex that is expressed at levels sufficient for its biophysical characterization.

1/3,AB/2
DIALOG(R)File 155:MEDLINE(R)


10771314 20058960 PMID: 10593387
Deglycosylation of a bifunctional lutropin-follitropin agonist reduced its follitropin activity more than its lutropin activity.
Trout SW; Han Y; Myers RV; Bernard MP; Moyle WR
Department of Obstetrics and Gynecology, Robert Wood Johnson (Rutgers) Medical School, Piscataway, New Jersey, USA.
Fertility and sterility (UNITED STATES) Dec 1999, 72 (6) p1093-9, ISSN 0015-0282 Journal Code: EVF
Contract/Grant No.: DK50600, DK, NIDDK; HD14907, HD, NICHD Languages: ENGLISH
Document type: Journal Article
Record type: Completed
OBJECTIVE: To design a drug that blocks the gonadal actions of lutropins and follitropins. DESIGN: Controlled in vitro study. SETTING: Academic laboratory. PATIENT(S): None. INTERVENTION(S): We removed three glycosylation signals from an hCG-hFSH chimera known to have high affinity for LH and FSH receptors, expecting this would create a bifunctional antagonist (dgCFC). To offset the inhibition of subunit combination caused by deglycosylation of alpha-subunit loop 2, we prepared dgCFC as a *single*-*chain* fusion protein containing the alpha-subunit downstream of the chimeric beta-subunit. MAIN OUTCOME MEASURE(S): Receptor binding, cyclic adenosine monophosphate accumulation. RESULT(S): dgCFC bound LH or FSH receptors similar to hCG or hFSH. It was a partial agonist and had one tenth the efficacy of hFSH and two thirds the efficacy of hCG. CONCLUSION(S): The surprising high residual lutropin activity of dgCFC indicated that its FSH residues offset the effects of deglycosylation, suggesting this approach to preparing a bifunctional antagonist is unlikely to lead to a useful drug. The increased lutropin efficacy of dgCFC relative to deglycosylated hCG supports the idea that oligosaccharides modulate *glycoprotein* *hormone* efficacy through an influence on hormone conformation.

1/3,AB/3
DIALOG(R)File 155:MEDLINE(R)


10436887 20065649 PMID: 10598590
The biological action of choriogonadotropin is not dependent on the complete native quaternary interactions between the subunits.
Jackson AM; Berger P; Pixley M; Klein C; Hsueh AJ; Boime I Department of Molecular Biology and Pharmacology, Washington University School of Medicine, St Louis, Missouri 63110, USA.
Molecular endocrinology (UNITED STATES) Dec 1999, 13 (12) p2175-88, ISSN 0888-8809 Journal Code: NGZ
Languages: ENGLISH
Document type: Journal Article
Record type: Completed
Human CG (hCG) is a member of the *glycoprotein* *hormone* family characterized by a heterodimeric structure consisting of a common alpha-subunit noncovalently bound to a hormone-specific beta-subunit. The two subunits are highly intertwined and only the heterodimer is functional, implying that the quaternary structure is critical for biological activity. To assess the dependence of the bioactivity of hCG on the heterodimeric interactions, alpha- and beta-subunits bearing mutations that prevent assembly were covalently linked to form a *single* *chain* hCG. Receptor binding and signal transduction of these analogs were tested and their structural integrity analyzed using a panel of monoclonal antibodies (mAbs). These included dimer-specific mAbs, which react with at least four different epitope sites on the hormone, and some that react only with the free beta-subunit. We showed that there was significant loss of quaternary and tertiary structure in several regions of the molecule. This was most pronounced in single chains that had one of the disulfide bonds of the cystine knot disrupted in either the alpha- or beta-subunit. Despite these structural changes, the in vitro receptor binding and signal transduction of the *single* *chain* analogs were comparable to those of the nonmutated *single* *chain* , demonstrating that not all of the quaternary configuration of the hormone is necessary for biological activity.

1/3,AB/4
DIALOG(R)File 155:MEDLINE(R)


10433919 20080261 PMID: 10614624
A biologically active *single* *chain* human chorionic gonadotropin analog with altered receptor binding properties.
Narayan P; Gray J; Puett D
Department of Biochemistry and Molecular Biology, University of Georgia, Athens 30602-7229, USA. narayan@bchiris.bmb.uga.edu
Endocrinology (UNITED STATES) Jan 2000, 141 (1) p67-71, ISSN 0013-7227 Journal Code: EGZ
Contract/Grant No.: DK-33973, DK, NIDDK
Languages: ENGLISH

Document type: Journal Article
Record type: Completed
hCG is a heterodimer consisting of an alpha-subunit common among all members of the *glycoprotein* *hormone* family, LH, FSH, and TSH, and a unique beta-subunit responsible for receptor specificity. Biologically active *single* *chain* analogs of these hormones have been engineered in which the C-terminus of the beta-subunit was fused to the N-terminus of the alpha-subunit (N-beta-alpha-C) either with or without a linker such as the hCGbeta C-terminal peptide (CTP). This tandem order of subunits was chosen based on studies suggesting that the N-terminal region of hCGbeta and particularly the C-terminal region of the alpha-subunit are important in receptor binding and activation. *Single* *chain* hCG (YhCG1) can, in turn, be fused to the LH receptor to yield a hormone-receptor complex that is biologically active in transfected cells. Herein, we report the construction of a new *single* *chain* hCG analog (YhCG3) in which the C-terminus of the alpha-subunit is fused to the N-terminus of hCGbeta via a CTP (N-alpha-CTP-beta-C). Compared with YhCG1, this analog binds receptor with a 25- to 30-fold lower affinity, but, surprisingly, is capable of stimulating intracellular cAMP levels to the same extent. Furthermore, YhCG3 can be covalently linked to its receptor to produce a biologically active complex that results in elevated levels of basal cAMP in transfected cells. These results suggest that free N- and C-termini of hCGbeta and the alpha-subunit, respectively, are not essential for receptor binding and activation and that YhCG3 is in a more efficacious conformation for receptor activation than YhCG1.

1/3,AB/5
DIALOG(R)File 155:MEDLINE(R)


10411096 20016596 PMID: 10548880
*Glycoprotein* *hormone* structure-function and analog design. Boime I; Ben-Menahem D
Department of Molecular Biology and Pharmacology, Washington University School of Medicine, St. Louis, Missouri 63110, USA.
Recent progress in hormone research (UNITED STATES) 1999, 54 p271-88; discussion 288-9, ISSN 0079-9963 Journal Code: R1D
Languages: ENGLISH
Document type: Journal Article; Review; Review, Tutorial
Record type: Completed
Human chorionic gonadotropin (hCG), luteinizing hormone, follicle-stimulating hormone (FSH), and thyrotropin (TSH) are hormones that share a common alpha subunit but differ in their beta subunits. Recombinant DNA techniques, valuable tools for structure-function analyses, provide an approach for designing therapeutic analogs. FSH is used clinically to stimulate the ovarian follicles for in vitro fertilization and to initiate follicular maturation in women with infertility problems. The CG beta subunit contains a carboxy-terminal extension (CTP) with four serine O-linked oligosaccharides, which is important for the long half-life of hCG. A clinical problem of FSH is its relatively short half-life in circulation. Fusing CTP to the FSH beta coding sequence increased the in vivo potency of the resulting FSH dimer over three-fold. Analogs of the other hormones containing CTP also increase their biologic half-life. Subunit assembly is vital to the function of these hormones. To address whether alpha and beta subunits can be synthesized as one chain and also maintain biological activity, a chimera comprised of the hCG beta subunit genetically fused to the alpha subunit was constructed. The resulting polypeptide was efficiently secreted and displayed an increased biologic activity in vitro and in vivo. Similarly, the *single*-*chain* form of FSH also retained in vivo activity. Since subunit dissociation inactivates the activity of the heterodimer, *single*-*chain* analogs should have longer biological half-lives. These analogs represent suitable substrates for engineering potent and stable agonists and antagonists.

1/3,AB/6
DIALOG(R)File 155:MEDLINE(R)


09659924 98113153 PMID: 9442031
Thyrotropin receptor cleavage at site 1 does not involve a specific amino acid motif but instead depends on the presence of the unique, 50 amino acid insertion.
Tanaka K; Chazenbalk GD; McLachlan SM; Rapoport B
Thyroid Molecular Biology Unit, Veterans Affairs Medical Center, San Francisco, California, USA.
Journal of biological chemistry (UNITED STATES) Jan 23 1998, 273 (4) p1959-63, ISSN 0021-9258 Journal Code: HIV
Contract/Grant No.: DK19289, DK, NIDDK; DK48216, DK, NIDDK Languages: ENGLISH
Document type: Journal Article
Record type: Completed
Thyrotropin (TSH) receptor (TSHR) A and B subunits are formed by intramolecular cleavage of the *single* *chain* receptor at two separate sites. The region involved in cleavage at Site 2 has been identified, but previous mutagenesis studies failed to identify Site 1. We now report fortuitous observations on the effect of trypsin on the TSHR that localizes a small region harboring Site 1. Thus, as detected by immunoblotting and by 125I-TSH cross-linking to TSHR expressed on the surface of intact CHO cells,

trypsin clipped a small polypeptide fragment bearing a glycan moiety from the C terminus of the A subunit. Based on the TSHR primary structure, this small fragment (1-2 kDa) contains Asn-302. This information, together with estimation of the size of the deglycosylated A subunit relative to a series of C-terminal truncated TSHR ectodomain variants, places cleavage Site 1 in the vicinity of, or closely upstream to, residue 317. Remarkably, mutagenesis of every amino acid residue between residues 298-316 (present study) and 317-362 (previous data) did not prevent cleavage at Site 1. However, cleavage at this site was abrogated by deletion of a 50-amino acid segment (residues 317-366) unique to the TSHR in the *glycoprotein* *hormone* receptor family. In summary, these data provide novel insight into TSHR intramolecular cleavage. Cleavage at Site 1 does not depend on a specific amino acid motif and differs from cleavage at Site 2 by involvement of a mechanism requiring the presence of the enigmatic TSHR 50-amino acid "insertion."

1/3,AB/7
DIALOG(R)File 155:MEDLINE(R)


09553483 97407919 PMID: 9261143
Human thyroid-stimulating hormone (hTSH) subunit gene fusion produces hTSH with increased stability and serum half-life and compensates for mutagenesis-induced defects in subunit association.
Grossmann M; Wong R; Szkudlinski MW; Weintraub BD
Department of Medicine, University of Maryland School of Medicine and the Institute of Human Virology, Medical Biotechnology Center, Baltimore, Maryland 21201, USA. grossman@umbi.umd.edu
Journal of biological chemistry (UNITED STATES) Aug 22 1997, 272 (34) p21312-6, ISSN 0021-9258 Journal Code: HIV
Languages: ENGLISH
Document type: Journal Article
Record type: Completed
The human thyroid-stimulating hormone (hTSH) subunits alpha and beta are transcribed from different genes and associate noncovalently to form the bioactive hTSH heterodimer. Dimerization is rate-limiting for hTSH secretion, and dissociation leads to hormone inactivation. Previous studies on human chorionic gonadotropin (hCG) and human follicle-stimulating hormone had shown that it was possible by subunit gene fusion to produce a bioactive, *single* *chain* hormone. However, neither the stability nor the clearance from the circulation of such fused glycoprotein hormones has been studied. We show here that genetic fusion of the hTSH alpha- and beta-subunits using the carboxyl-terminal peptide of the hCG beta-subunit as a linker created unimolecular hTSH whose receptor binding and bioactivity were comparable to native hTSH. Interestingly, the fused hTSH had higher thermostability and a longer plasma half-life than either native or dimeric hTSH containing the hCG beta-subunit-carboxyl-terminal peptide, suggesting that dimer dissociation may contribute to *glycoprotein* *hormone* inactivation in vivo. In addition, we show for the first time that synthesis of hTSH as a single polypeptide chain could overcome certain mutagenesis-induced defects in hTSH secretion, therefore enabling functional studies of such mutants. Thus, in addition to prolongation of plasma half-life, genetic fusion of hTSH subunits should be particularly relevant for the engineering of novel analogs where desirable features are offset by decreased dimer formation or stability. Such methods provide a general approach to expand the spectrum of novel recombinant glycoprotein hormones available for in vitro and in vivo study.

1/3,AB/8
DIALOG(R)File 155:MEDLINE(R)


09506163 96192928 PMID: 8614408
Functional expression of yoked human chorionic gonadotropin in baculovirus-infected insect cells.
Narayan P; Wu C; Puett D
Department of Biochemistry and Molecular Biology, University of Georgia, Athens 30602, USA.
Molecular endocrinology (UNITED STATES) Dec 1995, 9 (12) p1720-6, ISSN 0888-8809 Journal Code: NGZ
Contract/Grant No.: DK-33973, DK, NIDDK
Languages: ENGLISH
Document type: Journal Article
Record type: Completed
hCG is a *glycoprotein* *hormone* composed of an alpha-subunit, common to all gonadotropins and to TSH, and a hormone-specific beta-subunit. The non-covalent association of the two subunits is an obligatory step for the formation of biologically active hormones. The correct assembly of the heterodimer is also important for efficient secretion of the hormone, receptor binding, and signal transduction. Herein, we have demonstrated that expression of the two subunits from independent promoters present in a single recombinant baculovirus resulted in subunit association and secretion of biologically active holoprotein by the insect cells. To determine whether the active conformation of heterodimer could be achieved when the two subunits were synthesized in tandem on a single polypeptide chain, two *single* *chain* or yoked hCG1, the C-terminus of the complete beta-subunit (145 amino acid residues) was conjoined to the N-terminus of the alpha-subunit. Yoked hCG2 was similar, except that it contained the N-terminal 123 amino acid residues of the

beta-subunit. Both yoked hCG molecules bound LH/CG receptor with high affinity and stimulated adenylate cyclase and progesterone levels in transformed mouse Leydig (MA-10) cells. Therefore, the alpha- and beta-subunits are able to fold into a biologically active conformation when covalently linked. Interestingly, when compared with urinary hCG, the hormone expressed in baculovirus-infected insect cells binds to the LH/CG receptor with higher affinity, but exhibits diminished signaling, thus providing another example of a partial dissociation between receptor binding and activation.

1/3,AB/9
DIALOG(R)File 155:MEDLINE(R)
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09076900 97094949 PMID: 8940183
Protein engineering of a novel constitutively active hormone-receptor complex.
Wu C; Narayan P; Puett D
Department of Biochemistry and Molecular Biology, University of Georgia, Athens, Georgia 30602-7229, USA.
Journal of biological chemistry (UNITED STATES) Dec 6 1996, 271 (49) p31638-42, ISSN 0021-9258 Journal Code: HIV
Contract/Grant No.: DK-33973, DK, NIDDK
Languages: ENGLISH
Document type: Journal Article
Record type: Completed
Human chorionic gonadotropin (hCG) is a heterodimeric *glycoprotein* *hormone* consisting of an alpha and a beta subunit that stimulates intracellular levels of cAMP via a G protein-coupled receptor. Herein we report the engineering and characterization of a novel molecule in which the receptor and its heterodimeric ligand were covalently linked in a single polypeptide chain. The hormone-receptor complex was expressed in cells transfected with this construct, but the cells were unable to bind significant amounts of exogenous hCG. However, cleavage of the hormone with a site-specific protease rendered the receptor accessible to exogenously added hormone. Cells transfected with the hCG-receptor construct contained elevated basal levels of cAMP; moreover, addition of hormone had no significant effect. These results are consistent with a strong and stable interaction between the *single*-*chain* hormone and its covalently linked receptor that results in a constitutively active complex.

1/3,AB/10
DIALOG(R)File 155:MEDLINE(R)
(c) format only 2001 Dialog Corporation. All rts. reserv.

08794365 95199288 PMID: 7892221
Biosynthesis of a biologically active single peptide chain containing the human common alpha and chorionic gonadotropin beta subunits in tandem. Sugahara T; Pixley MR; Minami S; Perlas E; Ben-Menahem D; Hsueh AJ; Boime I
Department of Molecular Biology and Pharmacology, Washington University School of Medicine, St. Louis, MO 63110.
Proceedings of the National Academy of Sciences of the United States of America (UNITED STATES) Mar 14 1995, 92 (6) p2041-5, ISSN 0027-8424 Journal Code: PV3
Contract/Grant No.: N01-HD92922, HD, NICHD
Languages: ENGLISH
Document type: Journal Article
Record type: Completed
One of the distinguishing features of the gonadotropin and thyrotropin hormone family is their heterodimeric structure, consisting of a common alpha subunit and a hormone-specific beta subunit. Subunit assembly is vital to the function of these hormones: The conformation of the heterodimer is essential for controlling secretion, hormone-specific posttranslational modifications, and signal transduction. To address whether alpha and beta subunits can be synthesized as one chain and also maintain biological activity, a chimera composed of the human chorionic gonadotropin (hCG) beta subunit genetically fused to the alpha subunit was constructed. The resulting polypeptide hCG molecule not only was efficiently secreted but also displayed an increased biological activity in vitro and in vivo. These data show that the alpha and hCG beta subunits encoded as a *single* *chain* retain a biologically active conformation similar to that seen in the heterodimer. This approach can be used to investigate structure-function relationships of the *glycoprotein* *hormone* family that were previously not tractable because of the absolute dependence on assembly for the biological response. Moreover, other bioactive multisubunit ligands can be engineered where the combination efficiency and specificity of heterodimers and homodimers are otherwise difficult to control.

```
? s glycoprotein()hormone and yoked

      59620 GLYCOPROTEIN
     175585 HORMONE
        743 GLYCOPROTEIN(W)HORMONE
        384 YOKED
S2        3 GLYCOPROTEIN()HORMONE AND YOKED
? s s2 not s1

          3 S2
         10 S1
S3        0 S2 NOT S1
? b 411
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24jul01 11:43:59 User217743 Session D534.3
$2.22 0.695 DialUnits File155
$0.00 1 Type(s) in Format 8
$2.00 10 Type(s) in Format 4 (UDF)
$2.00 11 Types
$4.22 Estimated cost File155
$0.15 TYMNET
$4.37 Estimated cost this search
$4.37 Estimated total session cost 0.924 DialUnits
File 411:DIALINDEX(R)

DIALINDEX(R)
(c) 2001 The Dialog Corporation plc

*** DIALINDEX search results display in an abbreviated *** *** format unless you enter the SET DETAIL ON command. *** ? set files biochem

>>> 162 is unauthorized
>>>1 of the specified files is not available
You have 25 files in your file list.
(To see banners, use SHOW FILES command)
? s glycoprotein()hormone and (single()chain or yoked)

Your SELECT statement is:
s glycoprotein()hormone and (single()chain or yoked)

| Items | File |
|---|---|
| 13 | 5: Biosis Previews(R)_1969-2001/Jul W3 |
| 18 | 34: SciSearch(R) Cited Ref Sci_1990-2001/Jul W4 |
| 7 | 71: ELSEVIER BIOBASE_1994-2001/Jul W4 |
| 10 | 73: EMBASE_1974-2001/Jul W3 |
| 2 | 76: Life Sciences Collection_1982-2001/May |
| 1 | 98: General Sci Abs/Full-Text_1984-2001/Jun |
| 3 | 144: Pascal_1973-2001/Jul W4 |
| 10 | 155: MEDLINE(R)_1966-2001/Jul W5 |
| 5 | 156: Toxline(R)_1965-2000/Dec |
| 1 | 172: EMBASE Alert_2001/Jul W4 |
| 1 | 399: CA SEARCH(R)_1967-2001/UD=13504 |

11 files have one or more items; file list includes 25 files.
? rf

Your last SELECT statement was:
S GLYCOPROTEIN()HORMONE AND (SINGLE()CHAIN OR YOKED)

| Ref | Items | File |
|---|---|---|
| N1 | 18 | 34: SciSearch(R) Cited Ref Sci_1990-2001/Jul W4 |
| N2 | 13 | 5: Biosis Previews(R)_1969-2001/Jul W3 |
| N3 | 10 | 73: EMBASE_1974-2001/Jul W3 |
| N4 | 10 | 155: MEDLINE(R)_1966-2001/Jul W5 |
| N5 | 7 | 71: ELSEVIER BIOBASE_1994-2001/Jul W4 |
| N6 | 5 | 156: Toxline(R)_1965-2000/Dec |
| N7 | 3 | 144: Pascal_1973-2001/Jul W4 |
| N8 | 2 | 76: Life Sciences Collection_1982-2001/May |
| N9 | 1 | 98: General Sci Abs/Full-Text_1984-2001/Jun |
| N10 | 1 | 172: EMBASE Alert_2001/Jul W4 |

11 files have one or more items; file list includes 25 files.
- Enter P or PAGE for more -
? b n4, n1

24jul01 11:45:26 User217743 Session D534.4
$1.78 1.422 DialUnits File411
$1.78 Estimated cost File411
$0.10 TYMNET
$1.88 Estimated cost this search
$6.25 Estimated total session cost 2.346 DialUnits
SYSTEM:OS - DIALOG OneSearch
File 155:MEDLINE(R) 1966-2001/Jul W5
(c) format only 2001 Dialog Corporation
*File 155: This file has been reloaded. Accession numbers have changed. Please see Help News155 for further details.
File 34:SciSearch(R) Cited Ref Sci 1990-2001/Jul W4
(c) 2001 Inst for Sci Info

| Set | Items | Description |
|---|---|---|
| ? s glycoprotein()hormone and (single()chain or yoked) | | |
| | 132373 | GLYCOPROTEIN |
| | 330303 | HORMONE |
| | 1741 | GLYCOPROTEIN(W)HORMONE |
| | 996149 | SINGLE |
| | 569092 | CHAIN |
| | 9387 | SINGLE(W)CHAIN |
| | 620 | YOKED |
| S1 | 28 | GLYCOPROTEIN()HORMONE AND (SINGLE()CHAIN OR YOKED) ? rd |

...completed examining records
S2 19 RD (unique items)
? t s2/3,ab/all

2/3,AB/1 (Item 1 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
(c) format only 2001 Dialog Corporation. All rts. reserv.

11221856 21150064 PMID: 11250932
High-level expression of a functional *single*-*chain*

human chorionic gonadotropin-luteinizing hormone receptor ectodomain complex in insect cells.
Fralish GB; Narayan P; Puett D
Department of Biochemistry and Molecular Biology, University of Georgia, Athens, Georgia 30602-7229, USA.
Endocrinology (United States) Apr 2001, 142 (4) p1517-24, ISSN 0013-7227 Journal Code: EGZ
Contract/Grant No.: DK-33973, DK, NIDDK
Languages: ENGLISH
Document type: Journal Article
Record type: Completed
Reproductive capacity in primates is dependent on the high-affinity binding of the glycoprotein hormones LH and human (h)CG to the large ectodomain (ECD) of their common receptor (LHR). Our understanding of the precise molecular determinants of hormone binding is limited, because there are no structural data for any of the *glycoprotein* *hormone* receptors. Overexpression of the ECD of the receptor has been attempted in various expression systems. Prokaryotic expression does not yield properly folded ECD. Eukaryotic expression, on the other hand, results in mostly heterogeneous, intracellularly trapped protein, but the secreted ECD is completely folded. Accordingly, we have tethered the *single*- *chain* hormone, *yoked* hCG, to the N terminus of LHR-ECD ( *yoked* hormone-extracellular domain). *Yoked* hCG is secreted at high levels; binds LHR with high affinity; and, when tethered to the N terminus of full-length LHR, it binds and constitutively activates the receptor. Using recombinant baculovirus, *yoked* hormone-extracellular domain is secreted from insect cells at levels greater than 1 microg/ml, nearly 20-fold higher than that previously reported in eukaryotic expression systems. The protein was purified and binds exogenous (125)I-hCG with high affinity but, significantly, only after protease treatment to remove the tethered hormone. Thus, the fusion protein seems to form a functional hormone-receptor complex that is expressed at levels sufficient for its biophysical characterization.

2/3,AB/2 (Item 2 from file: 155)
DIALOG(R)File 155:MEDLINE(R)


10771314 20058960 PMID: 10593387
Deglycosylation of a bifunctional lutropin-follitropin agonist reduced its follitropin activity more than its lutropin activity.
Trout SW; Han Y; Myers RV; Bernard MP; Moyle WR
Department of Obstetrics and Gynecology, Robert Wood Johnson (Rutgers) Medical School, Piscataway, New Jersey, USA.
Fertility and sterility (UNITED STATES) Dec 1999, 72 (6) p1093-9, ISSN 0015-0282 Journal Code: EVF
Contract/Grant No.: DK50600, DK, NIDDK; HD14907, HD, NICHD Languages: ENGLISH
Document type: Journal Article
Record type: Completed
OBJECTIVE: To design a drug that blocks the gonadal actions of lutropins and follitropins. DESIGN: Controlled in vitro study. SETTING: Academic laboratory. PATIENT(S): None. INTERVENTION(S): We removed three glycosylation signals from an hCG-hFSH chimera known to have high affinity for LH and FSH receptors, expecting this would create a bifunctional antagonist (dgCFC). To offset the inhibition of subunit combination caused by deglycosylation of alpha-subunit loop 2, we prepared dgCFC as a *single*-*chain* fusion protein containing the alpha-subunit downstream of the chimeric beta-subunit. MAIN OUTCOME MEASURE(S): Receptor binding, cyclic adenosine monophosphate accumulation. RESULT(S): dgCFC bound LH or FSH receptors similar to hCG or hFSH. It was a partial agonist and had one tenth the efficacy of hFSH and two thirds the efficacy of hCG. CONCLUSION(S): The surprising high residual lutropin activity of dgCFC indicated that its FSH residues offset the effects of deglycosylation, suggesting this approach to preparing a bifunctional antagonist is unlikely to lead to a useful drug. The increased lutropin efficacy of dgCFC relative to deglycosylated hCG supports the idea that oligosaccharides modulate *glycoprotein* *hormone* efficacy through an influence on hormone conformation.

2/3,AB/3 (Item 3 from file: 155)
DIALOG(R)File 155:MEDLINE(R)


10436887 20065649 PMID: 10598590
The biological action of choriogonadotropin is not dependent on the complete native quaternary interactions between the subunits. Jackson AM; Berger P; Pixley M; Klein C; Hsueh AJ; Boime I Department of Molecular Biology and Pharmacology, Washington University School of Medicine, St Louis, Missouri 63110, USA.
Molecular endocrinology (UNITED STATES) Dec 1999, 13 (12) p2175-88, ISSN 0888-8809 Journal Code: NGZ
Languages: ENGLISH
Document type: Journal Article
Record type: Completed
Human CG (hCG) is a member of the *glycoprotein* *hormone* family characterized by a heterodimeric structure consisting of a common alpha-subunit noncovalently bound to a hormone-specific beta-subunit. The two subunits are highly intertwined and only the heterodimer is functional, implying that the quaternary structure is critical for biological activity. To assess the

dependence of the bioactivity of hCG on the heterodimeric interactions, alpha- and beta-subunits bearing mutations that prevent assembly were covalently linked to form a *single* *chain* hCG. Receptor binding and signal transduction of these analogs were tested and their structural integrity analyzed using a panel of monoclonal antibodies (mAbs). These included dimer-specific mAbs, which react with at least four different epitope sites on the hormone, and some that react only with the free beta-subunit. We showed that there was significant loss of quaternary and tertiary structure in several regions of the molecule. This was most pronounced in single chains that had one of the disulfide bonds of the cystine knot disrupted in either the alpha- or beta-subunit. Despite these structural changes, the in vitro receptor binding and signal transduction of the *single* *chain* analogs were comparable to those of the nonmutated *single* *chain* , demonstrating that not all of the quaternary configuration of the hormone is necessary for biological activity.

2/3,AB/4 (Item 4 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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10433919 20080261 PMID: 10614624
A biologically active *single* *chain* human chorionic gonadotropin analog with altered receptor binding properties. Narayan P; Gray J; Puett D
Department of Biochemistry and Molecular Biology, University of Georgia, Athens 30602-7229, USA. narayan@bchiris.bmb.uga.edu
Endocrinology (UNITED STATES) Jan 2000, 141 (1) p67-71, ISSN 0013-7227 Journal Code: EGZ
Contract/Grant No.: DK-33973, DK, NIDDK
Languages: ENGLISH
Document type: Journal Article
Record type: Completed
hCG is a heterodimer consisting of an alpha-subunit common among all members of the *glycoprotein* *hormone* family, LH, FSH, and TSH, and a unique beta-subunit responsible for receptor specificity. Biologically active *single* *chain* analogs of these hormones have been engineered in which the C-terminus of the beta-subunit was fused to the N-terminus of the alpha-subunit (N-beta-alpha-C) either with or without a linker such as the hCGbeta C-terminal peptide (CTP). This tandem order of subunits was chosen based on studies suggesting that the N-terminal region of hCGbeta and particularly the C-terminal region of the alpha-subunit are important in receptor binding and activation. *Single* *chain* hCG (YhCG1) can, in turn, be fused to the LH receptor to yield a hormone-receptor complex that is biologically active in transfected cells. Herein, we report the construction of a new *single* *chain* hCG analog (YhCG3) in which the C-terminus of the alpha-subunit is fused to the N-terminus of hCGbeta via a CTP (N-alpha-CTP-beta-C). Compared with YhCG1, this analog binds receptor with a 25- to 30-fold lower affinity, but, surprisingly, is capable of stimulating intracellular cAMP levels to the same extent. Furthermore, YhCG3 can be covalently linked to its receptor to produce a biologically active complex that results in elevated levels of basal cAMP in transfected cells. These results suggest that free N- and C-termini of hCGbeta and the alpha-subunit, respectively, are not essential for receptor binding and activation and that YhCG3 is in a more efficacious conformation for receptor activation than YhCG1.

2/3,AB/5 (Item 5 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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10411096 20016596 PMID: 10548880
*Glycoprotein* *hormone* structure-function and analog design. Boime I; Ben-Menahem D
Department of Molecular Biology and Pharmacology, Washington University School of Medicine, St. Louis, Missouri 63110, USA.
Recent progress in hormone research (UNITED STATES) 1999, 54 p271-88; discussion 288-9, ISSN 0079-9963 Journal Code: R1D
Languages: ENGLISH
Document type: Journal Article; Review; Review, Tutorial
Record type: Completed
Human chorionic gonadotropin (hCG), luteinizing hormone, follicle-stimulating hormone (FSH), and thyrotropin (TSH) are hormones that share a common alpha subunit but differ in their beta subunits. Recombinant DNA techniques, valuable tools for structure-function analyses, provide an approach for designing therapeutic analogs. FSH is used clinically to stimulate the ovarian follicles for in vitro fertilization and to initiate follicular maturation in women with infertility problems. The CG beta subunit contains a carboxy-terminal extension (CTP) with four serine O-linked oligosaccharides, which is important for the long half-life of hCG. A clinical problem of FSH is its relatively short half-life in circulation. Fusing CTP to the FSH beta coding sequence increased the in vivo potency of the resulting FSH dimer over three-fold. Analogs of the other hormones containing CTP also increase their biologic half-life. Subunit assembly is vital to the function of these hormones. To address whether alpha and beta subunits can be synthesized as one chain and also maintain biological activity, a chimera comprised of the hCG beta subunit genetically fused to the alpha subunit was constructed. The resulting

polypeptide was efficiently secreted and displayed an increased biologic activity in vitro and in vivo. Similarly, the *single*-*chain* form of FSH also retained in vivo activity. Since subunit dissociation inactivates the activity of the heterodimer, *single*-*chain* analogs should have longer biological half-lives. These analogs represent suitable substrates for engineering potent and stable agonists and antagonists.

2/3,AB/6 (Item 6 from file: 155)
DIALOG(R)File 155:MEDLINE(R)


09659924 98113153 PMID: 9442031
Thyrotropin receptor cleavage at site 1 does not involve a specific amino acid motif but instead depends on the presence of the unique, 50 amino acid insertion.
Tanaka K; Chazenbalk GD; McLachlan SM; Rapoport B
Thyroid Molecular Biology Unit, Veterans Affairs Medical Center, San Francisco, California, USA.
Journal of biological chemistry (UNITED STATES) Jan 23 1998, 273 (4) p1959-63, ISSN 0021-9258 Journal Code: HIV
Contract/Grant No.: DK19289, DK, NIDDK; DK48216, DK, NIDDK Languages: ENGLISH
Document type: Journal Article
Record type: Completed
Thyrotropin (TSH) receptor (TSHR) A and B subunits are formed by intramolecular cleavage of the *single* *chain* receptor at two separate sites. The region involved in cleavage at Site 2 has been identified, but previous mutagenesis studies failed to identify Site 1. We now report fortuitous observations on the effect of trypsin on the TSHR that localizes a small region harboring Site 1. Thus, as detected by immunoblotting and by 125I-TSH cross-linking to TSHR expressed on the surface of intact CHO cells, trypsin clipped a small polypeptide fragment bearing a glycan moiety from the C terminus of the A subunit. Based on the TSHR primary structure, this small fragment (1-2 kDa) contains Asn-302. This information, together with estimation of the size of the deglycosylated A subunit relative to a series of C-terminal truncated TSHR ectodomain variants, places cleavage Site 1 in the vicinity of, or closely upstream to, residue 317. Remarkably, mutagenesis of every amino acid residue between residues 298-316 (present study) and 317-362 (previous data) did not prevent cleavage at Site 1. However, cleavage at this site was abrogated by deletion of a 50-amino acid segment (residues 317-366) unique to the TSHR in the *glycoprotein* *hormone* receptor family. In summary, these data provide novel insight into TSHR intramolecular cleavage. Cleavage at Site 1 does not depend on a specific amino acid motif and differs from cleavage at Site 2 by involvement of a mechanism requiring the presence of the enigmatic TSHR 50-amino acid "insertion."

2/3,AB/7 (Item 7 from file: 155)
DIALOG(R)File 155:MEDLINE(R)


09553483 97407919 PMID: 9261143
Human thyroid-stimulating hormone (hTSH) subunit gene fusion produces hTSH with increased stability and serum half-life and compensates for mutagenesis-induced defects in subunit association.
Grossmann M; Wong R; Szkudlinski MW; Weintraub BD
Department of Medicine, University of Maryland School of Medicine and the Institute of Human Virology, Medical Biotechnology Center, Baltimore, Maryland 21201, USA. grossman@umbi.umd.edu
Journal of biological chemistry (UNITED STATES) Aug 22 1997, 272 (34) p21312-6, ISSN 0021-9258 Journal Code: HIV
Languages: ENGLISH
Document type: Journal Article
Record type: Completed
The human thyroid-stimulating hormone (hTSH) subunits alpha and beta are transcribed from different genes and associate noncovalently to form the bioactive hTSH heterodimer. Dimerization is rate-limiting for hTSH secretion, and dissociation leads to hormone inactivation. Previous studies on human chorionic gonadotropin (hCG) and human follicle-stimulating hormone had shown that it was possible by subunit gene fusion to produce a bioactive, *single* *chain* hormone. However, neither the stability nor the clearance from the circulation of such fused glycoprotein hormones has been studied. We show here that genetic fusion of the hTSH alpha- and beta-subunits using the carboxyl-terminal peptide of the hCG beta-subunit as a linker created unimolecular hTSH whose receptor binding and bioactivity were comparable to native hTSH. Interestingly, the fused hTSH had higher thermostability and a longer plasma half-life than either native or dimeric hTSH containing the hCG beta-subunit-carboxyl-terminal peptide, suggesting that dimer dissociation may contribute to *glycoprotein* *hormone* inactivation in vivo. In addition, we show for the first time that synthesis of hTSH as a single polypeptide chain could overcome certain mutagenesis-induced defects in hTSH secretion, therefore enabling functional studies of such mutants. Thus, in addition to prolongation of plasma half-life, genetic fusion of hTSH subunits should be particularly relevant for the engineering of novel analogs where desirable features are offset by decreased dimer formation or stability. Such methods provide a general approach to expand the spectrum of novel recombinant glycoprotein hormones available for in vitro and in vivo study.

2/3,AB/8 (Item 8 from file: 155)
DIALOG(R)File 155:MEDLINE(R)


09506163 96192928 PMID: 8614408
Functional expression of *yoked* human chorionic gonadotropin in baculovirus-infected insect cells.
Narayan P; Wu C; Puett D
Department of Biochemistry and Molecular Biology, University of Georgia, Athens 30602, USA.
Molecular endocrinology (UNITED STATES) Dec 1995, 9 (12) p1720-6, ISSN 0888-8809 Journal Code: NGZ
Contract/Grant No.: DK-33973, DK, NIDDK
Languages: ENGLISH
Document type: Journal Article
Record type: Completed
hCG is a *glycoprotein* *hormone* composed of an alpha-subunit, common to all gonadotropins and to TSH, and a hormone-specific beta-subunit. The non-covalent association of the two subunits is an obligatory step for the formation of biologically active hormones. The correct assembly of the heterodimer is also important for efficient secretion of the hormone, receptor binding, and signal transduction. Herein, we have demonstrated that expression of the two subunits from independent promoters present in a single recombinant baculovirus resulted in subunit association and secretion of biologically active holoprotein by the insect cells. To determine whether the active conformation of heterodimer could be achieved when the two subunits were synthesized in tandem on a single polypeptide chain, two *single* *chain* or *yoked* hCG1, the C-terminus of the complete beta-subunit (145 amino acid residues) was conjoined to the N-terminus of the alpha-subunit. *Yoked* hCG2 was similar, except that it contained the N-terminal 123 amino acid residues of the beta-subunit. Both *yoked* hCG molecules bound LH/CG receptor with high affinity and stimulated adenylate cyclase and progesterone levels in transformed mouse Leydig (MA-10) cells. Therefore, the alpha- and beta-subunits are able to fold into a biologically active conformation when covalently linked. Interestingly, when compared with urinary hCG, the hormone expressed in baculovirus-infected insect cells binds to the LH/CG receptor with higher affinity, but exhibits diminished signaling, thus providing another example of a partial dissociation between receptor binding and activation.

2/3,AB/9 (Item 9 from file: 155)
DIALOG(R)File 155:MEDLINE(R)


09076900 97094949 PMID: 8940183
Protein engineering of a novel constitutively active hormone-receptor complex.
Wu C; Narayan P; Puett D
Department of Biochemistry and Molecular Biology, University of Georgia, Athens, Georgia 30602-7229, USA.
Journal of biological chemistry (UNITED STATES) Dec 6 1996, 271 (49) p31638-42, ISSN 0021-9258 Journal Code: HIV
Contract/Grant No.: DK-33973, DK, NIDDK
Languages: ENGLISH
Document type: Journal Article
Record type: Completed
Human chorionic gonadotropin (hCG) is a heterodimeric *glycoprotein* *hormone* consisting of an alpha and a beta subunit that stimulates intracellular levels of cAMP via a G protein-coupled receptor. Herein we report the engineering and characterization of a novel molecule in which the receptor and its heterodimeric ligand were covalently linked in a single polypeptide chain. The hormone-receptor complex was expressed in cells transfected with this construct, but the cells were unable to bind significant amounts of exogenous hCG. However, cleavage of the hormone with a site-specific protease rendered the receptor accessible to exogenously added hormone. Cells transfected with the hCG-receptor construct contained elevated basal levels of cAMP; moreover, addition of hormone had no significant effect. These results are consistent with a strong and stable interaction between the *single*-*chain* hormone and its covalently linked receptor that results in a constitutively active complex.

2/3,AB/10 (Item 10 from file: 155)
DIALOG(R)File 155:MEDLINE(R)


08794365 95199288 PMID: 7892221
Biosynthesis of a biologically active single peptide chain containing the human common alpha and chorionic gonadotropin beta subunits in tandem. Sugahara T; Pixley MR; Minami S; Perlas E; Ben-Menahem D; Hsueh AJ; Boime I
Department of Molecular Biology and Pharmacology, Washington University School of Medicine, St. Louis, MO 63110.
Proceedings of the National Academy of Sciences of the United States of America (UNITED STATES) Mar 14 1995, 92 (6) p2041-5, ISSN 0027-8424 Journal Code: PV3
Contract/Grant No.: N01-HD92922, HD, NICHD
Languages: ENGLISH
Document type: Journal Article
Record type: Completed
One of the distinguishing features of the gonadotropin and thyrotropin hormone family is their heterodimeric structure, consisting of a common alpha subunit and a hormone-specific beta subunit. Subunit assembly is vital

to the function of these hormones: The conformation of the heterodimer is essential for controlling secretion, hormone-specific posttranslational modifications, and signal transduction. To address whether alpha and beta subunits can be synthesized as one chain and also maintain biological activity, a chimera composed of the human chorionic gonadotropin (hCG) beta subunit genetically fused to the alpha subunit was constructed. The resulting polypeptide hCG molecule not only was efficiently secreted but also displayed an increased biological activity in vitro and in vivo. These data show that the alpha and hCG beta subunits encoded as a *single* *chain* retain a biologically active conformation similar to that seen in the heterodimer. This approach can be used to investigate structure-function relationships of the *glycoprotein* *hormone* family that were previously not tractable because of the absolute dependence on assembly for the biological response. Moreover, other bioactive multisubunit ligands can be engineered where the combination efficiency and specificity of heterodimers and homodimers are otherwise difficult to control.

2/3,AB/11 (Item 1 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci
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05981519 Genuine Article#: XL735 Number of References: 43 Title: Cystine knot of the gonadotropin alpha subunit is critical for intracellular behavior but not for in vitro biological activity ( ABSTRACT AVAILABLE)
Author(s): Sato A; Perlas E; BenMenahem D; Kudo M; Pixley MR; Furuhashi M; Hsueh AJW; Boime I (REPRINT)
Corporate Source: WASHINGTON UNIV,SCH MED, DEPT MOL BIOL & PHARMACOL, 660 S EUCLID AVE, BOX 8103/ST LOUIS//MO/63110 (REPRINT); WASHINGTON UNIV,SCH MED, DEPT MOL BIOL & PHARMACOL/ST LOUIS//MO/63110; STANFORD UNIV,MED CTR, DIV REPROD BIOL, DEPT OBSTET GYNECOL/STANFORD//CA/94305 Journal: JOURNAL OF BIOLOGICAL CHEMISTRY, 1997, V272, N29 (JUL 18), P 18098-18103
ISSN: 0021-9258 Publication date: 19970718
Publisher: AMER SOC BIOCHEMISTRY MOLECULAR BIOLOGY INC, 9650 ROCKVILLE PIKE, BETHESDA, MD 20814
Language: English Document Type: ARTICLE
Abstract: The common alpha subunit of glycoprotein hormones contains five disulfide bonds. Based on the published crystal structure, the assignments are 7-31, 59-87, 10-60, 25-82, and 32-84; the last three comprise the cystine knot, a structure also seen in a variety of growth factors, Previously, we demonstrated that the efficiency of secretion and the ability to form heterodimers by alpha subunits hearing single cysteine residue mutants in the cystine knot were significantly reduced. These results suggested that the cystine knot is critical for the intracellular integrity of the subunit, To assess if the presence of the free thiol. affected the secretion kinetics, we constructed paired cysteine mutants of each disulfide bond of the alpha subunit. The secretion rate for these monomers was comparable with mild type except for the alpha-10-60 mutant, which was 40% lower. The recovery of the alpha 7-31 and alpha 59-87 mutants was greater than 95%, whereas for the cystine knot mutants, it was 20-40%. Co-expression of the wild-type chorionic gonadotropin beta subunit with double cysteine mutants did not enhance the recovery of alpha mutants in the media, Moreover, compared with wild-type, the efficiency of heterodimer formation of the alpha 10-60 or alpha 32-84 mutants was less than 5%. Because subunit assembly is required for biological activity, steadies oil the role of these disulfide bonds in signal transduction mere not possible. To bypass the assembly step, we exploited the *single* *chain* model, where the alpha and beta subunits are genetically fused. The recovery of secreted tethered gonadotropins bearing mutations in the cystine knot was increased significantly. Although dimer-specific monoclonal antibodies discriminated the conformation of *single* *chain* alpha 10-60 and alpha 32-84 mutants from the native heterodimer, these mutants were nevertheless biologically active. Thus, individual bonds of cystine knot are important for secretion and heteradimer formation but not for in vitro bioactivity. Moreover, the data suggest that the native heterodimer configuration is not a prerequisite for receptor binding or signal transduction.

2/3,AB/12 (Item 2 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci
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05932380 Genuine Article#: XH583 Number of References: 39 Title: Design of stable biologically active recombinant lutropin analogs ( ABSTRACT AVAILABLE)
Author(s): GarciaCampayo V; Sato A; Hirsch B; Sugahara T; Muyan M; Hsueh AJW; Boime I (REPRINT)
Corporate Source: WASHINGTON UNIV,SCH MED, DEPT MOL BIOL & PHARMACOL, 660 S EUCLID AVE, BOX 8103/ST LOUIS//MO/63110 (REPRINT); WASHINGTON UNIV,SCH MED, DEPT MOL BIOL & PHARMACOL/ST LOUIS//MO/63110; STANFORD UNIV,MED CTR, DIV REPROD BIOL, DEPT OBSTET GYNECOL/STANFORD//CA/94305 Journal: NATURE BIOTECHNOLOGY, 1997, V15, N7 (JUL), P663-667
ISSN: 1087-0156 Publication date: 19970700

Publisher: NATURE PUBLISHING CO, 345 PARK AVE SOUTH, NEW YORK, NY 10010-1707
Language: English Document Type: ARTICLE
Abstract: Glycoprotein hormones are noncovalent heterodimers comprised of a common alpha subunit and a hormone-specific beta subunit, Secretion and biologic action of these hormones are dependent on the formation of the heterodimer. The human LH beta subunit is unique among the other beta subunits in that it assembles inefficiently with the alpha subunit. To bypass this rate-limiting step, we constructed the LH single chains where the carboxy terminus of beta was fused to the amino terminus of alpha subunit through a linker. Compared to the human LH heterodimer, the extent of secretion was greater for the tethers although the rate was dependent on the nature of the linker. The LH single chains were biologically active even though there was loss of recognition by a LH-specific monoclonal antibody. This suggests that receptor binding of the single chains is not impaired by changes in the heterodimeric configuration resulting from tethering the subunits. In addition, single chains exhibited a remarkably greater in vitro stability than the heterodimer, implying that these analogs will be useful as diagnostic reagents and that their purification will be facilitated.

2/3,AB/13 (Item 3 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci
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05801886 Genuine Article#: WY448 Number of References: 25 Title: Evaluation of subunit truncation and the nature of the spacer for *single* *chain* human gonadotropins (ABSTRACT AVAILABLE) Author(s): Heikoop JC; vanBeuningendeVaan MMJACM; vandenBoogaart P; Grootenhuis PDJ (REPRINT) Corporate Source: NV ORGANON,DEPT COMPUTAT MED CHEM, SCI DEV GRP, POB 20/NL-5340 BH OSS//NETHERLANDS/ (REPRINT); NV ORGANON,DEPT COMPUTAT MED CHEM, SCI DEV GRP/NL-5340 BH OSS//NETHERLANDS/
Journal: EUROPEAN JOURNAL OF BIOCHEMISTRY, 1997, V245, N3 (MAY 1), P656-662 ISSN: 0014-2956 Publication date: 19970501
Publisher: SPRINGER VERLAG, 175 FIFTH AVE, NEW YORK, NY 10010 Language: English Document Type: ARTICLE
Abstract: Three *single* *chain* gonadotropins were designed based on the three-dimensional-structure of human choriogonadotropin and structure/activity relationships of the glycoprotein hormones. In each *single* *chain*, the C-terminal end of the human choriogonadotropin beta subunit is connected via Ser-Gly repeats to the N-terminal end of the alpha subunit. In addition, two of the single chains have truncated subunits. The three mutants were expressed in CHO cells. In vitro binding of two of the three mutants to the human lutropin/ choriogonadotropin receptor was found to be comparable to wild-type lutropin. In contrast, both the receptor binding and the in vitro bioactivity of the mutant with truncated alpha and beta subunits in which the beta:26-110 disulphide bond cannot be formed, are lowered relative to wild-type lutropin. The fact that this mutant still displays biological activity shows that the seat-belt arrangement proposed by Isaacs and coworkers [Lapthorn, A. J., Harris, D. C., Littlejohn, A., Lustbader, J. W., Canfield, R. E., Machin, K. J., Morgan, F. J. & Isaacs, N. W (1994) Nature 369, 455-461] is important but not essential for receptor binding and biological activity in the context of *single* *chain* gonadotropins. Single chains in which Ser-Gly spacers are combined with truncated subunits, provide an attractive approach towards the design and generation of novel, biologically active gonadotropins.

2/3,AB/14 (Item 4 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci
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05695058 Genuine Article#: WQ589 Number of References: 24 Title: Characterization of the carbohydrate structures of apolipoprotein H through concanavalin A affinity chromatography (ABSTRACT AVAILABLE) Author(s): Gambino R; Ruiu G; Pagano G; Cassader M (REPRINT) Corporate Source: UNIV TURIN,DIPARTIMENTO MED INTERNA, CORSO AM DOGLIOTTI 14/I-10126 TURIN//ITALY/ (REPRINT); UNIV TURIN,DIPARTIMENTO MED INTERNA/I-10126 TURIN//ITALY/; AZIENDA OSPED S GIOVANNI BATTISTA,LAB CENT BALDI & RIBERI/TURIN//ITALY/
Journal: JOURNAL OF LIPID MEDIATORS AND CELL SIGNALLING, 1997, V16, N1 (MAY ), P11-21
ISSN: 0929-7855 Publication date: 19970500
Publisher: ELSEVIER SCIENCE BV, PO BOX 211, 1000 AE AMSTERDAM, NETHERLANDS Language: English Document Type: ARTICLE
Abstract: Apolipoprotein H, also known as beta 2-Glycoprotein I, is a *single* *chain* highly glycosylated polypeptide of 326 amino acids. The carbohydrate content of apolipoprotein H is approximately 19% of the molecular weight. Some studies have described the main oligosaccharides forming the glycosylated chains but the carbohydrate inner structures of apolipoprotein H has not been investigated yet. This gap should be filled being glycosylation a very important process which is able to regulate the structure and the biological functions of proteins. Lectins are proteins which specifically bind carbohydrate structures. Affinity chromatography of

glycoproteins on immobilized lectins, such as Concanavalin A (Con A), has been proved to be a useful method for oligosaccharide fractionation. N-Linked oligosaccharide structures were shown to interact with Con A according to their branching properties. In the present study, we analyzed the patterns of Con A elution of apolipoprotein H isolated from human plasma. Using Con A affinity chromatography we show that apolipoprotein H has a high degree of heterogeneity in its glycosylated structure. It allowed one to isolate two groups of apolipoprotein H molecules bearing biantennary and truncated hybrids and high mannose and hybrid oligosaccharides. Since Con A affinity chromatography allows fractionation of molecules differing in the extent of carbohydrate branching irrespective of the sialyl residues, we can conclude that mannose residues are masked with other galactose-beta (1-4)N-acetyl-glucosamine, galactose-beta (1-3)Ai-acetyl-galactosamine and sialic acid linked alpha(2-6) to galactose or to N-acetylgalactosamine, or capped with sulfated residues. Thus, according to our results apolipoprotein H presents truncated hybryd or hybrid-type carbohydrate chains which bear few unmasked mannose residues as terminal sugar. Moreover, isoelectrofocusing of apolipoprotein H forms fractionated on Con A demostrates that weakly bound material presents a predominance of more acidic isoforms than that firmly bound to the lectin, indicating that weakly bound fractions contain molecules which are more negatively charged and that Con A is able to separate glycosylated forms which are not discriminated by isoelectrofocusing. (C) 1997 Elsevier Science B.V.

2/3,AB/15 (Item 5 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci
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05648627 Genuine Article#: WN147 Number of References: 29 Title: The biologic action of *single*-*chain* choriogonadotropin is not dependent on the individual disulfide bonds of the beta subunit (ABSTRACT AVAILABLE)
Author(s): BenMenahem D; Kudo M; Pixley MB; Sato A; Suganuma N; Perlas E; Hsueh AJW; Boime I (REPRINT)
Corporate Source: WASHINGTON UNIV,SCH MED, DEPT MOL BIOL & PHARMACOL/ST LOUIS//MO/63110 (REPRINT); WASHINGTON UNIV,SCH MED, DEPT MOL BIOL & PHARMACOL/ST LOUIS//MO/63110; STANFORD UNIV,MED CTR, DEPT OBSTET GYNECOL, DIV REPROD BIOL/STANFORD//CA/94305
Journal: JOURNAL OF BIOLOGICAL CHEMISTRY, 1997, V272, N11 (MAR 14), P 6827-6830
ISSN: 0021-9258 Publication date: 19970314
Publisher: AMER SOC BIOCHEMISTRY MOLECULAR BIOLOGY INC, 9650 ROCKVILLE PIKE, BETHESDA, MD 20814
Language: English Document Type: ARTICLE
Abstract: Disrupting disulfide loops in the human chorionic gonadotropin beta subunit (CG beta) inhibits combination with the alpha subunit. Because the bioactivity requires a heterodimer, studies on the role of disulfide bonds on receptor binding/signal transduction have previously been precluded. To address this problem, we bypassed the assembly step and genetically fused CGP subunits bearing paired cysteine mutations to a wild-type a (WT alpha) subunit. The changes altered secretion of the *single*-*chain* mutants which parallel that seen for the CGP monomeric subunit. Despite conformational changes in CG disulfide bond mutants (assayed by gel electrophoresis and conformationally sensitive monoclonal antibodies), the variants bind to the lutropin/CG receptor and activated adenylate cyclase in vitro. The data show that the structural requirements for secretion and bioactivity are not the same. The results also suggest that the extensive native subunit interactions determined by the cystine bonds are not required for signal transduction. Moreover, these studies demonstrate that the *single* -*chain* model is an effective approach to structure-activity relationships of residues and structural domains associated with assembly of multisubunit ligands.

2/3,AB/16 (Item 6 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci
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05507911 Genuine Article#: WC923 Number of References: 27 Title: Expression of biologically active fusion genes encoding the common alpha subunit and either the CG beta or FSH beta subunits: Role of a linker sequence (ABSTRACT AVAILABLE)
Author(s): Sugahara T; Grootenhuis PDJ; Sato A; Kudo M; BenMenahem D; Pixley MR; Hsueh AJW; Boime I (REPRINT)
Corporate Source: WASHINGTON UNIV,SCH MED, DEPT MOL BIOL & PHARMACOL, 600 S EUCLID AVE, BOX 8103/ST LOUIS//MO/63110 (REPRINT); WASHINGTON UNIV,SCH MED, DEPT MOL BIOL & PHARMACOL/ST LOUIS//MO/63110; NV ORGANON,DEPT COMPUTAT MED CHEM/NL-5340 BH OSS//NETHERLANDS/; STANFORD UNIV,MED CTR, DEPT GYNAECOL OBSTET, DIV REPROD BIOL/STANFORD//CA/94305 Journal: MOLECULAR AND CELLULAR ENDOCRINOLOGY, 1996, V125, N1-2 (DEC 20), P 71-77
ISSN: 0303-7207 Publication date: 19961220
Publisher: ELSEVIER SCI IRELAND LTD, CUSTOMER

RELATIONS MANAGER, BAY 15, SHANNON INDUSTRIAL ESTATE CO, CLARE, IRELAND
Language: English Document Type: ARTICLE
Abstract: The gonadotropin/thyrotropin hormone family is characterized by a heterodimeric structure composed of a common alpha subunit non-covalently linked toa hormone-specific beta subunit. The conformation of the heterodimer is essential for controlling secretion, hormone-specific post-translational modifications and signal transduction. Structure-function studies of FSH and the other glycoprotein hormones are often hampered by mutagenesis induced defects in subunit combination. Thus, the ability to overcome the limitation of subunit assembly would expand the range of structure activity relationships that can be performed on these hormones. Here we converted the FSH heterodimer to a *single* *chain* by genetically fusing the carboxyl end of the FSH beta subunit to the amino end of the alpha subunit in the presence or absence of a natural linker sequence. In the absence of the CTP linker, the secretion rate was decreased over three fold. (The CTP sequence is the last 28 amino acids of the CG beta sequence and contains four serine-linked oligosaccharides). Unexpectedly however, receptor binding/signal transduction was unaffected by absence of the linker. Molecular modelling of the tethers lacking the linker sequence show that the alignment of the alpha/beta domains in the *single* *chain* differ substantially from that seen in the heterodimer.These data show that the *single* *chain* FSH was secreted efficiently and is biologically active and that the conformation determinants required for secretion and biologic activity are not the same. Copyright (C) 1996 Elsevier Science Ireland Ltd.

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05507909 Genuine Article#: WC923 Number of References: 61 Title: hCG-receptor binding and transmembrane signaling (ABSTRACT AVAILABLE)
Author(s): Puett D (REPRINT) ; Bhowmick N; Fernandez LM; Huang JN; Wu CB; Narayan P
Corporate Source: UNIV GEORGIA,DEPT BIOCHEM & MOL BIOL/ATHENS//GA/30602 (REPRINT)
Journal: MOLECULAR AND CELLULAR ENDOCRINOLOGY, 1996, V125, N1-2 (DEC 20), P 55-64
ISSN: 0303-7207 Publication date: 19961220
Publisher: ELSEVIER SCI IRELAND LTD, CUSTOMER RELATIONS MANAGER, BAY 15, SHANNON INDUSTRIAL ESTATE CO, CLARE, IRELAND
Language: English Document Type: ARTICLE
Abstract: The technique of site-directed mutagenesis has proven to be quite powerfulin elucidating contact sites involved in the interaction of the heterodimeric glycoprotein hormones and their respective seven transmembrane (TM) G protein-coupled receptors. Our laboratory has focused on identification of the minimum core sequences of the alpha and beta subunits required for bioactivity, the minimum length of a conjoined (*yoked*) *single*-*chain* hCG, the amino acid residues on hCG and the LH/CG-receptor (LH/CG-R) responsible for high-affinity binding, and the regions of the receptor that are involved in TM signaling. A number of amino acid residues have been mapped on the alpha and beta subunits of hCG that appear important in receptor binding. When projected onto the crystal structure of HF-treated hCG, these residues, by and large, cluster on one side of the molecule and cover a sizeable surface area, indicating that the hormone-receptor binding interface is rather extensive. Based on mutagenesis studies of several conserved ionizable amino acid residues in the extracellular domain (ECD) of LH/CG-R and a model that we, in collaboration with Drs Lapthorn and Isaacs, have developed for this region based on the crystal structure of porcine ribonuclease inhibitor, a charged region that appears to play an important role in hormone-receptor recognition has been identified. We have also delineated several regions of LH/CG-R that do not appear to participate in hCG binding but are involved in hCG-mediated signaling. These regions are located in the ECD and extracellular loop III just prior to entry into the membrane via TM helices I and VII: respectively, and in TM helices VI and VII. Similarly, a homologous region in the ECD of the FSH receptor, located with ten residues of TM helix I, is important in signaling but not hormone binding. These results suggest that ligand binding and ligand-mediated receptor activation are quasi-distinct, albeit sequential phenomena. Collectively, our mutagenesis and modeling studies, coupled with results from other laboratories, argue for a ligand-induced conformational change of the receptor that may involve a relative reorientation of the TM helices. Copyright (C) 1996 Elsevier Science Ireland Ltd.

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03846809 Genuine Article#: QM408 Number of References: 36 Title: BIOSYNTHESIS OF A BIOLOGICALLY-ACTIVE SINGLE PEPTIDE-CHAIN CONTAINING THE HUMAN COMMON ALPHA-SUBUNITS AND CHORIONIC-GONADOTROPIN BETA-SUBUNITS IN TANDEM (Abstract Available)

Author(s): SUGAHARA T; PIXLEY MR; MINAMI S; PERLAS E; BENMENAHEM D; HSUEH AJW; BOIME I
Corporate Source: WASHINGTON UNIV,SCH MED,DEPT MOLEC BIOL & PHARMACOL,660 S EUCLID AVE,BOX 8103/ST LOUIS//MO/63110; STANFORD UNIV,MED CTR,DEPT OBSTET GYNECOL,DIV REPROD BIOL/STANFORD//CA/94305
Journal: PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, 1995, V92, N6 (MAR 14), P2041-2045 ISSN: 0027-8424
Language: ENGLISH Document Type: ARTICLE
Abstract: One of the distinguishing features of the gonadotropin and thyrotropin hormone family is their heterodimeric structure, consisting of a common alpha subunit and a hormone-specific beta subunit. Subunit assembly is vital to the function of these hormones: The conformation of the heterodimer is essential for controlling secretion, hormone-specific posttranslational modifications, and signal transduction. To address whether alpha and beta subunits can be synthesized as one chain and also maintain biological activity, a chimera composed of the human chorionic gonadotropin (hCG) beta subunit genetically fused to the alpha subunit was constructed. The resulting polypeptide hCG molecule not only was efficiently secreted but also displayed an increased biological activity in vitro and in vivo. These data show that the alpha and hCG beta subunits encoded as a *single* *chain* retain a biologically active conformation similar to that seen in the heterodimer. This approach can be used to investigate structure-function relationships of the *glycoprotein* *hormone* family that were previously not tractable because of the absolute dependence on assembly for the biological response. Moreover, other bioactive multisubunit ligands can be engineered where the combination efficiency and specificity of heterodimers and homodimers are otherwise difficult to control.

2/3,AB/19 (Item 9 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci
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02863757 Genuine Article#: MK939 Number of References: 44 Title: COMPARISON OF THE CYTOTOXIC EFFECT OF HORMONOTOXINS PREPARED WITH THE USE OF HETEROBIFUNCTIONAL CROSS-LINKING AGENTS N-SUCCINIMIDYL 3-(2-PYRIDYLDITHIO)PROPIONATE AND N-SUCCINIMIDYL

6-[3-(2-PYRIDYLDITHIO)PROPIONAMIDO]HEXANOAT E (Abstract Available) Author(s): SINGH V; MAVILA AK; KAR SK
Corporate Source: NE HILL UNIV,INST SELF ORGANISING SYST & BIOPHYS,PERMANENT CAMPUS/SHILLONG 793022/MEGHALAYA/INDIA/; JAWAHARLAL NEHRU UNIV,CTR BIOTECHNOL/NEW DELHI 110067//INDIA/
Journal: BIOCONJUGATE CHEMISTRY, 1993, V4, N6 (NOV-DEC), P473-482 ISSN: 1043-1802
Language: ENGLISH Document Type: ARTICLE
Abstract: With the aim of developing cytotoxic hybrid molecules which can be selectively targeted to specific cells in the gonads, a *single* *chain* ribosome-inactivating protein, gelonin, was conjugated to ovine luteinizing hormone (oLH) with the use of heterobifunctional cross-linking agents N-succinimidyl 3-(2-pyridyldithio)-propionate (SPDP) and long-chain SPDP. Four hormonotoxins were synthesized having a variable spacer arm between oLH and gelonin. The spacer arms in C200A, C210A, C220A, and C230A were 13.6, 22.4, 22.4, and 31.2 angstrom long, respectively. Extensive physicochemical and biochemical analysis revealed a 1:1 molar ratio of the ingredients in its oLH-S-S-gelonin conjugates. The linkage occurred through the epsilon-NH2 group of the alpha-subunit of oLH as judged from RP-HPLC analysis. The hormonotoxins retained substantial receptor binding ability, steriodogenic activity, and immunoreactivity of oLH and gelonin to their respective antibodies. Hormonotoxins bind to Leydig tumor cells via oLH, leaving gelonin free as judged by competitive displacement analysis. The hormonotoxins internalized to a sufficient degree to effectively inhibit protein synthesis. Upon comparison, immunoreactivity, receptor binding steroidogenic activity, and cytotoxicity of oLH-S-S-gelonin conjugates prepared with the use of only LC-SPDP (C230A, 31.2-angstrom spacer arm) and by using both SPDP and LC-SPDP (C210A and C220A, 22.4-A spacer arm) were found to be comparable with that of conjugate prepared with SPDP alone (C200A, 13.6-angstrom spacer arm). Therefore, it may be concluded that the cytotoxicity of oLH-based hormonotoxin remained unaffected with the use of long-chain spacer arms which are believed to be used generally to avoid steric hindrance.
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